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Case Research

Recurrent isochromosome 21 and multiple abnormalities in a patient suspected of having acute myeloid leukemia with eosinophilic differentiation—a rare case from South India

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Abstract

Acute myeloid leukemia (AML) is a phenotypically heterogeneous disorder. The M4 subtype of AML is frequently associated with the cytogenetic marker inversion 16 and/or the presence of eosinophilia. Blast crisis is the aggressive phase of the triphasic chronic myeloid leukemia (CML), which is a disease with Philadelphia (Ph) chromosome as the major abnormality. In the present study, we report a 76-year-old patient suspected of having AML with eosinophilic differentiation (AML-M4), which in clinical tests resembles CML blast crisis with multiple chromosomal abnormalities. Isochromosome 21 [i(21)(q10)] was the most recurrent feature noted in metaphases with 46 chromosomes. Ring chromosome, tetraploid endoreduplication, recurrent aneuploid clones with loss of X chromosome, monosomy 17, monosomy 7, and structural variation translocation (9;14) were also observed in this patient. Fluorescent *in situ* hybridization (FISH) confirmed the absence of Ph chromosome. This report shows how cytogenetic analyses revealed atypical structural aberrations in the M4 subtype of AML.

Key words: i(21)(q10), AML-M4 [E0], atypical cytogenetic abnormalities, tetraploid endoreduplication, ring chromosome

Acute myelogenous leukemia (AML) is a hematopoietic malignancy, with the accumulation of immature cells in the marrow, peripheral blood, and eventually other tissues. Primary chromosomal abnormalities in AML are highly specific and considered to be associated with leukemic transformation, whereas secondary changes are less specific and probably contribute to disease progression. The common chromosomal abnormalities in the French-American-British Cooperative Group (FAB) subtype M4, or acute myelomonocytic leukemia, include monosomy 5 or deletion of 5q [del(5q)], monosomy 7 or del(7q), trisomy 8, t(6;9)(p23;q34), and others. Karyotype is generally an important prognostic factor in AML, with prognosis being associated with even minor karyotypic changes. In this report, we describe an adult male patient diagnosed with hematological and clinical characteristics of AML-M4 with eosinophilic differentiation (AML-M4 [E0]) showing atypical cytogenetic features.

Case Presentation

A 76-year-old man of Indian origin, with history of ischemic heart disease, was presented to the Outpatient Clinic of Medical Oncology, Regional Cancer Centre due to marked variation in blood count, in addition to anemia, thrombocytopenia, mild splenomegaly, and leukocytosis. Initial complete blood count displayed 4.8 g/dL of hemoglobin, leukocyte count of 50 500 cells/mm^3 undiluted blood, and thrombocyte count of 65 000 cells/mm^3 undiluted blood. Blood picture showed predominant neutrophils, marked shift to the left with atypical monocytes, 12% blasts, and 6% basophils. Initial bone marrow study revealed hypercellular marrow with 35% blasts and mild

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eosinophilia. Blasts were peroxidase-positive. The hematological and clinical parameters were consistent with AML-M4 [E0] that resembles blast crisis of chronic myeloid leukemia (CML). Using conventional cytogenetics and fluorescence *in situ* hybridization (FISH), the case was confirmed not to be CML blast crisis. Using standard protocols, unstimulated bone marrow culture and chromosome analysis were performed on G-banded metaphases using Cytovision software. The karyotypes were prepared according to the International System for Human Cytogenetic Nomenclature (2005). FISH was performed using BCR/ABL Dual Color, Dual Fusion Translocation Probe (Vysis). All studies were carried out after obtaining written informed consent from the patient and approval from the Institutional Review Board of the Regional Cancer Centre. Cytogenetic analysis using G-banding revealed the karyotypes in 50 intact metaphases, and the results are summarized in Table 1. In addition, rare phenomena including tetraploid endoreduplication and ring chromosomes were noted.

Discussion

Isochromosome forms when one arm of a chromosome is lost and the remaining arm is duplicated, resulting in a chromosome consisting of only two short arms or two long arms. Isochromosomes have rarely been reported as acquired aberrations in hematological malignancies, except in some survey data that shows the presence of isochromosome 21 [i(21)(q10)] in hematological malignancies and 10% of all neoplasms with cytogenetic aberrations[1]. Worth *et al.*[2] has reported a transient leukemic condition in a phenotypically normal newborn bearing i(21)(q10) clones, suggesting that the q arm of chromosome 21 contains sufficient genetic information for the development of transient leukemia. Among the common types of isochromsomes observed in AML, i(11q), i(17q), and i(21q) are the most prominent[3]. In our study, i(21)(q10) was the single recurrent abnormality observed in 84% (42/50) of the spreads (Figure 1A). It was either the sole abnormality (19/50 spreads) or present in conjunction with other

Table 1. Cytogenetic findings from 50 banded spreads

Composite karyotypes observed	No. of metaphases (n = 50)	Other abnormalities
46, XY	4	
46, XY, i(21)(q10)	19	
45, XY, −17, i(21)(q10)	2	
46, XY, −17, i(21)(q10)[cp6]	6	46, XY, +14, −17, i(21)(q10) 46, XY, −17, +F, i(21)(q10) 46, XY, +15, −17, i(21)(q10) 46, XY, −17, −19, +21, i(21)(q10), +22 46, XY, −17, +18, +19, i(21)(q10), −22 46, XY, −17, i(21)(q10), +mar
46, XY, −7, i(21)(q10)[cp4]	4	46, XY, −7, +E, i(21)(q10) 46, XY, −7, +19, i(21)(q10) 46, XY, −7, +D, i(21)(q10) 46, XY, −7, +14, i(21)(q10)
46, Y, −X, +14, −18, i(21)(q10)[cp3]	3	46, Y0, −X, +14, i(21)(q10) 46, Y0, −X, +17, −18, i(21)(q10), +22 46, Y0, −X, +14, −18, +19, i(21)(q10)
46, XY, i(21)(q10) [cp5]	5	46, XY, −16, +21, +21, i(21)(q10) 46, XY, +14, −18, i(21)(q10) 46, XY, +10, −12, i(21)(q10) 46, XY, +5, −8, i(21)(q10) 46, XY, +3, −11, i(21)(q10)
47, XY, +14[cp5]	5	47, XY, +14 47, XY, +18 47, XY, −C, +14, +15, +17, −21 47, XY, +C 47, XY, i(21)(q10), −B, +C, +mar
45, XY, i(21)(q10)[cp2]	2	45, XY, i(21)(q10), −22 45, XY, −19, i(21) (q10)

aneuploidies (23/50 spreads). A majority of the clones (84%) were cytogenetically related, as they were found to have i(21)(q10) and were derived from it. In spite of the presence of i(21)(q10) clones with dissimilar karyotypes, almost all clones retained 46 chromosomes.

An isochromosome has morphologically identical genetic information in both arms. The impact of gene dosage or copy number variations due to isochromosome formation in leukemia patients needs further attention. Recent reports indicate that *KCNE2* (potassium voltage-gated channel, Isk-related family, member 2), located on chromosome 21, is expressed in cardiac muscles and is involved in maintaining a regular cardiac rhythm by interacting with ion channels. The close relation of *KCNE2* with *KCNQ1* and *KCNE1* in cardiomyocytes has been noted to play a role in dynamic regulation of IKs (slow delayed rectifier IKs channels) current amplitude in the heart[4,5]. In our case study, the patient had ischemic heart disease, which might be inadvertently associated with *KCNE2* gene. The effect of its over-dose due to isochromosome formation needs detailed investigation. Furthermore, the load of aneuploid and tetraploid clones observed in our study might be associated to the increased copy number of pericentrin (*PCNT*), a gene located on chromosome 21 and produces pericentrin in AML. This observation is consistent with expression profiling data showing the overexpression of *PCNT*. Higher levels of pericentrin correlate with aneuploidy and centrosome aberration levels in AML[6,7]. Furthermore, Neben *et al.*[8] found that pericentrin is part of an expression signature identifying genes associated with tetraploidization in mantle cell lymphoma. Abnormal centrosomes are common in tumors and have strict correlation with cancer emergence[9]. Hence, it can be inferred that copy number and gene dosage variations due to increased levels of i(21)(q10) can be a clinical indicator for the pathophysiological manifestations of the disease stage and for prognosis.

Leukemia cells with either i(21)(q10) or trisomy 21 have the potential for basophil formation[2,10], consistent with our case in which basophils were detectable at basal levels. Miyauchi *et al.*[10] explained the involvement of GATA-binding factor 1 (*GATA1*) mutations in causing transient leukemia in cases with trisomy 21. Transient leukemia-related blasts have the potential to grow and differentiate towards particular hematopoietic lineages in the presence of specific hematopoietic growth factors. The down-regulation of *GATA1* might be involved in blast cell differentiation. In accordance with this, the status of mutations in *GATA1* in cases of i(21)(q10) warrants detailed investigation.

AML-M4 [E0] is a subtype of acute leukemia associated with an increase in eosinophils. Adriaanson *et al.*[11] found that chromosome 16 abnormalities linked to AML-M4 [E0] include inversion 16 [inv(16)], translocation (16;16) [t(16;16)], and deletion 16q22 [del(16q22)]. Several cytogenetic abnormalities have been reported in chronic eosinophilic leukemia, but there is no unique clonal cytogenetic abnormality associated with chronic eosinophilic leukemia. Del(16q22) was reported in only one chronic eosinophilic leukemia case and was associated with other chromosomal abnormalities, including trisomy 8, trisomy 19, and addition of 2q[12]. Notably, the patient subsequently developed AML. In our study, inv(16) and t(16;16) were not observed among the analyzed metaphases. However, the status of del (16q22) remains to be determined. Additional recurrent chromosomal abnormalities observed in the clones with i(21)(q10) included monosomy 17, monosomy 7, loss of X chromosomes, and others.

The second most common aneuploidy observed in our subject, occurring in an average of 16% of clones, was loss of a copy of chromosome 17. *p53* mutations were found more frequently in patients with monosomy 17p[13]. In addition, 17p anomaly-like monosomy 17/17p- has been observed in myelodysplastic syndrome and AML patients with poor prognosis and been found to occur as adjunct to secondary MDS/AML after chemotherapy and/or radiotherapy, usually in association with other complex chromosomal anomalies[14].

Monosomy 7 was also observed in several clones analyzed. An association between the complete or partial loss of chromosome 7 and preleukemic myelodysplasia or AML has been recognized from the early days of tumor cytogenetic analysis. Detection of such abnormalities usually heralds a poor prognosis[15]. Amare *et al.*[16] reported monosomy of chromosomes 7 and 17 as secondary chromosomal abnormalities that occur when disease progresses from CML to a more aggressive blastic phase or transforms into lymphoid leukemia-like acute myeloid, lymphoid leukemia, or lymphoid blast crisis of CML. Sabine *et al.*[17] have shown that monosomy 7/del(7q) causes loss of *miR-29a*, an important tumor suppressor, and up-regulation of *Ski* oncogene in AML. Zhang *et al.*[18] showed that benzene significantly induced monosomies of a specific set of chromosomes including chromosome 7 in a dose-dependent manner. Monosomy 7 as observed in our study has also been linked to loss of the chromosome 7 gene *KCNH2* [potassium voltage-gated channel, subfamily H (Eag-related), member 2], which was found to play a role in causing cardiac arrest death[19]. We hypothesize that some patients may show genomic imbalances and changes in the gene copy number that lead to genetic instability.

Bakshi *et al.*[20] reported the loss of sex chromosomes in AML and Philadelphia chromosome (Ph)−negative CML cases. Loss of X chromosome is purportedly due to evolution of malignant clones, but the influence of this loss on the process of evolution is not known. The loss of sex chromosomes has been shown to be clearly related to increasing age[21]. In our case, we also

observed X chromosome loss in several metaphases (6%).

Polyploidy and endoreduplication (P&E) were also observed in our study (Figure 1B). P&E of chromosomes occur more often in patients with disseminated cancer and vary with the extent of disease. Our data is substantiated by a prior report by Bottura *et al.*[22] that depicts the link between polyploidization in leukemia cells, specifically in AML. In our case, we observed ring chromosomes in 2 metaphases with 47 chromosomes (Figure 1C). It is probably not only the ring structure or the neoplastic nature of the host cell that determines ring instability, but also the function of the genes carried in the ring[23]. Structural variation t(9,14) in one of the metaphases (Figure 1D) was also a notable anomaly in our case. This type of translocation, t(9;14), has been detected in 50% of lymphocytoid lymphoma, a subtype of B-cell non-Hodgkin's lymphoma[24]. A similar case of t (9;14) among a complex karyotype has been reported in a patient with AML-M7[25]. Compared to other hematological malignancies, AML is frequently reported to have ring chromosomes[26]. Although certain clinical and

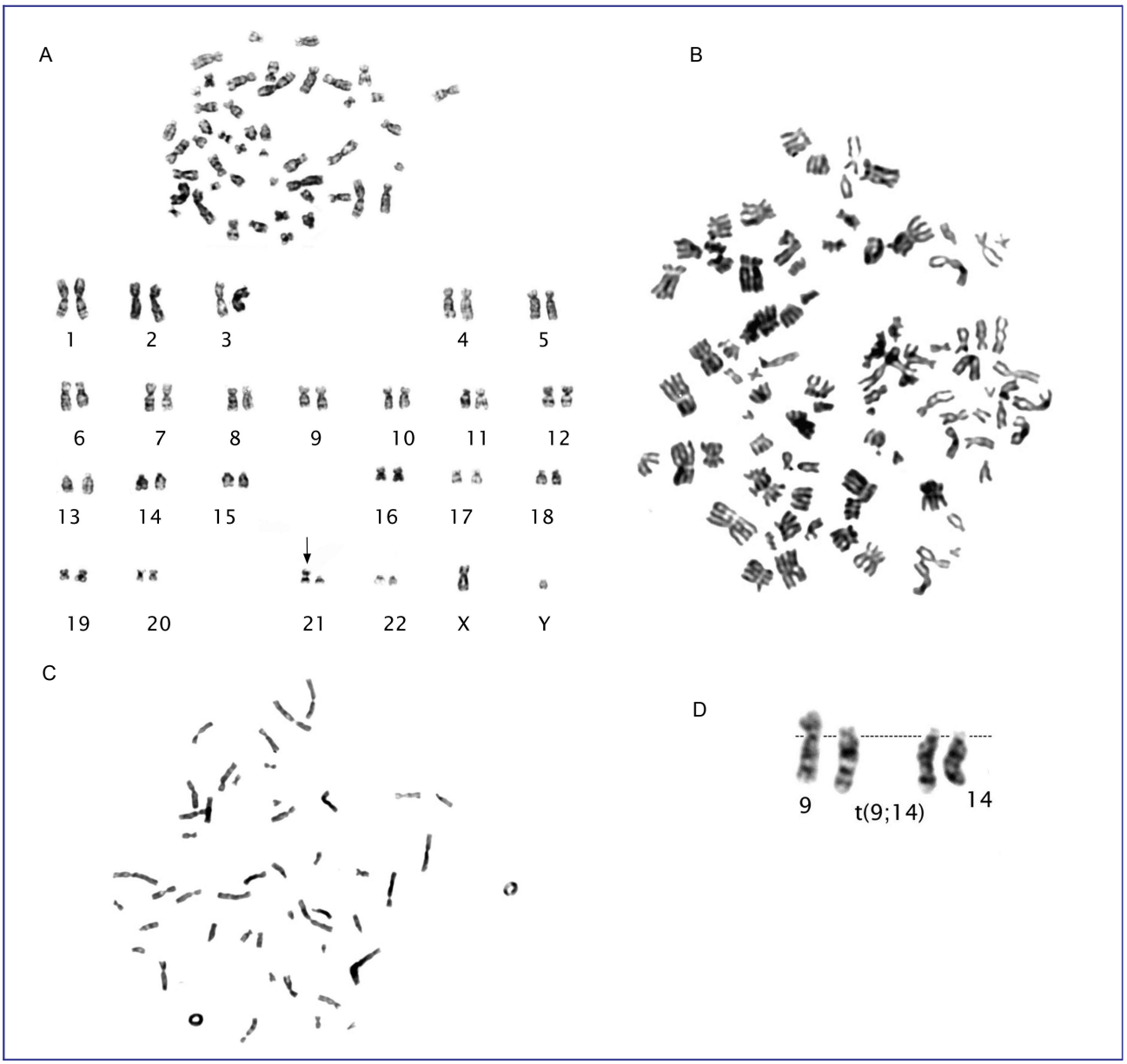

Figure 1. **Genotyping for a 76-year-old man with suspected AML-M4[EO].** A, G-banded metaphase and karyotype shows 46,XY,i(21)(q10). B, a metaphase shows tetraploid endoreduplication. C, a metaphase with 47 chromosomes shows two ring chromosomes. D, a partial karyotype of t(9;14).

hematological parameters of our case showed a slight inclination towards CML blast crisis, Ph chromosome was not observed by GTG-banding in any of the observed metaphases. To rule out the presence of any cryptic bcr-abl fusion gene, we performed FISH analysis (Figure 2), which confirmed the diagnosis of AML-M4 [E0].

Cytogenetic abnormalities play an important role in diagnosing hematological malignant diseases and are important independent predictors of disease progression and survival. Although karyotype is an important prognostic factor in AML, the prognostic significance of additional cytogenetic abnormalities like isochromosome 21 in acute leukemia remains to be elucidated. Atypical cytogenetic findings have been sporadically reported in AML-M4, but the scarcity of these abnormalities poses a challenge to using such changes as prognostic factors, reinforcing the need for the collection of clinical data on rare events. To the best of our knowledge, this is the first case report involving a spectrum of abnormalities associated with poor prognosis in AML-M4 [E0]. The atypical findings suggest a cumulative poor prognosis, and further follow-up of this patient is needed to justify this derivation.

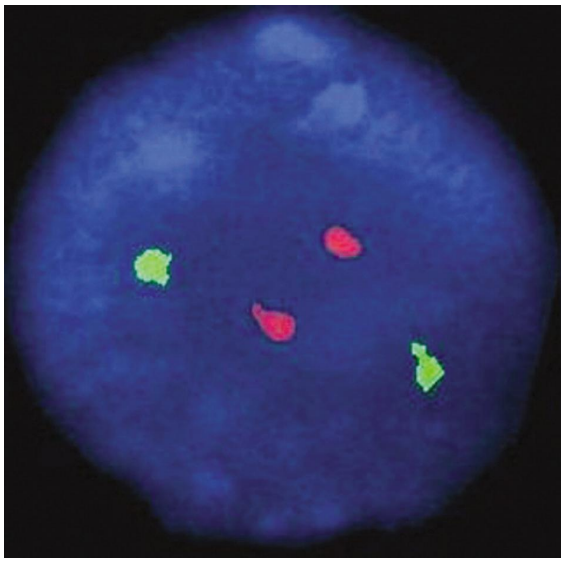

Figure 2. **An interphase FISH shows the absence of BCR-ABL fusion signal.** The two red signals indicate *ABL* gene on chromosome 9, and two green signals indicate *BCR* gene on chromosome 22.

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